

AD-A149 744

CULTURE METHODOLOGY FOR EXPERIMENTAL INVESTIGATIONS
INVOLVING ROOTED SUBM. (U) ARMY ENGINEER WATERWAYS
EXPERIMENT STATION VICKSBURG MS ENVIR.

1/1

UNCLASSIFIED

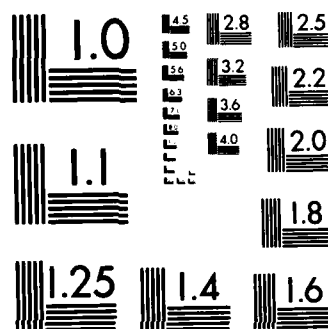
R M SMART ET AL. NOV 84 WES/MP/A-84-6

F/G 8/1

NI.

■

4





US Army Corps
of Engineers

AD-A149 744

AQUATIC PLANT CONTROL
RESEARCH PROGRAM

(12)

MISCELLANEOUS PAPER A-84-6

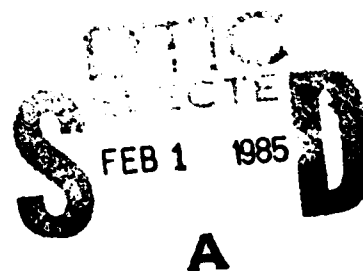
CULTURE METHODOLOGY FOR EXPERI-
MENTAL INVESTIGATIONS INVOLVING
ROOTED SUBMERSED AQUATIC PLANTS

by

R. Michael Smart, John W. Barko

Environmental Laboratory

DEPARTMENT OF THE ARMY
Waterways Experiment Station, Corps of Engineers
PO Box 631
Vicksburg, Mississippi 39180-0631



November 1984

Final Report

Approved For Public Release: Distribution Unlimited



Prepared for
DEPARTMENT OF THE ARMY
US Army Corps of Engineers
Washington, DC 20314-1000

85 01 24 089

Destroy this report when no longer needed. Do not return
it to the originator.

The findings in this report are not to be construed as an official
Department of the Army position unless so designated
by other authorized documents.

The contents of this report are not to be used for
advertising, publication, or promotional purposes.
Citation of trade names does not constitute an
official endorsement or approval of the use of
such commercial products.

Unclassified

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER Miscellaneous Paper A-84-6	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) CULTURE METHODOLOGY FOR EXPERIMENTAL INVESTIGATIONS INVOLVING ROOTED SUBMERSED AQUATIC PLANTS		5. TYPE OF REPORT & PERIOD COVERED Final report
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) R. Michael Smart, John W. Barko		8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS US Army Engineer Waterways Experiment Station Environmental Laboratory PO Box 631, Vicksburg, Mississippi 39180-0631		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS Aquatic Plant Control Research Program
11. CONTROLLING OFFICE NAME AND ADDRESS DEPARTMENT OF THE ARMY US Army Corps of Engineers Washington, DC 20314-1000		12. REPORT DATE November 1984
		13. NUMBER OF PAGES 20
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)		15. SECURITY CLASS. (of this report) Unclassified
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE
16. DISTRIBUTION STATEMENT (of this Report) Approved for public release; distribution unlimited.		
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)		
18. SUPPLEMENTARY NOTES Available from National Technical Information Service, 5285 Port Royal Road, Springfield, Virginia 22161.		
19. KEY WORDS (Continue on reverse side if necessary and identify by block number) Aquaculture Aquatic Plants		
20. ABSTRACT (Continue on reverse side if necessary and identify by block number) Recent information on the relative roles of sediment and water as nutrient sources for rooted submersed freshwater macrophytes has facilitated the development of a methodology for culturing these plants. The use of natural sediment as the source of nitrogen, phosphorus, and micronutrients, coupled with the omission of these nutrients from culture solution, largely prevents the occurrence of algal blooms and, for many purposes, obviates the use of elaborate axenic culturing techniques. (Continued)		

DD FORM 1 JAN 73 1473

EDITION OF 1 NOV 65 IS OBSOLETE

Unclassified

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

Unclassified

SECURITY CLASSIFICATION OF THIS PAGE(When Data Entered)

ABSTRACT (Continued).

Contemporary information on the growth requirements of submersed macrophytes is reviewed in relation to the provision of conditions suitable for the laboratory culture of these plants. Sediment substrate requirements are considered in relation to the role of sediment as a nutrient source. Two culture solution formulations are provided along with procedures for solution preparation. Procedures for establishing and maintaining cultures are also provided. The information presented is intended to be of assistance in the establishment and maintenance of submersed macrophyte cultures for experimental research.

Unclassified

SECURITY CLASSIFICATION OF THIS PAGE(When Data Entered)

Preface

The culture methodology presented in this report is based on contemporary information on the growth requirements of rooted submersed aquatic plants and incorporates pertinent findings from a number of investigations conducted in the US Army Engineer Waterways Experiment Station (WES) Environmental Laboratory (EL) between 1978 and 1984. Funding for these investigations was provided by the Office, Chief of Engineers (OCE), through the Aquatic Plant Control Research Program (APCRP) and the Environmental and Water Quality Operational Studies (EWQOS) Program. Technical Monitors for OCE during this study were Mr. E. Carl Brown for APCRP and Mr. Earl Eiker, Dr. John Bushman, and Mr. James L. Gottesman for EWQOS.

These investigations were performed under the general supervision of Dr. John Harrison, Chief, EL, and the direct supervision of Mr. Donald L. Robey, Chief, Ecosystem Research and Simulation Division (ERSD), WES, and Dr. Tom L. Hart, Chief, Aquatic Processes and Effects Group (APEG), ERSD. Authors of this report were Mr. R. Michael Smart and Dr. John W. Barko, APEG. Technical assistance was provided by Mr. Lee Ferguson, Ms. Susan Hennington, Ms. Dwillette McFarland, and Ms. Ramona Warren. Program Managers were Mr. J. Lewis Decell (APCRP) and Dr. Jerome L. Mahloch (EWQOS).

Commander and Director of WES during publication of this report was COL Robert C. Lee, CE. Technical Director was Mr. F. R. Brown.

This report should be cited as follows:

Smart, R. M., and Barko, J. W. 1984. "Culture Methodology for Experimental Investigations Involving Rooted Submersed Aquatic Plants," Miscellaneous Paper A-84-6, US Army Engineer Waterways Experiment Station, Vicksburg, Miss.

Contents

	<u>Page</u>
Preface	1
Introduction	3
Background	3
Objective and scope	3
Culture Requirements	4
Nutrient sources	4
Carbon supply and pH	6
Facilities	6
Culture Development	7
Culture solutions	7
Sediment substrate	9
Plant propagation	10
Culture Maintenance	11
Subculturing	11
Algal control	11
Maintenance of solution composition	12
Experimental Use of Cultures	13
References	14
Tables 1-2	

CULTURE METHODOLOGY FOR EXPERIMENTAL INVESTIGATIONS
INVOLVING ROOTED SUBMERSED AQUATIC PLANTS

Introduction

Background

1. One of the major problems of growing submersed aquatic plants in the laboratory is that of providing adequate nutrition while minimizing the growth of phytoplankton and attached algae, which, if uncontrolled, prohibits the development of macrophyte cultures (Gerloff and Krombholz 1966; Mulligan and Baranowski 1969; Ryan, Reimer, and Toth 1972; Mulligan, Baranowski, and Johnson 1976). Unfortunately, algae-free cultures are difficult to obtain (Wetzel and McGregor 1968) and require considerable care to prevent contamination over long periods of time (Denny 1980). In spite of the above problems, early efforts to culture submersed macrophytes were apparently influenced by the then widespread belief that most nutrients were obtained by shoot uptake from the water (see reviews by Bourn (1932) and Sculthorpe (1967)). Early culture media for submersed macrophytes were, therefore, understandably based almost exclusively on those developed for macrophytic algae (Pringsheim and Pringsheim 1962; Forsberg 1965; Imahori and Iwasa 1965) or hydroponic culture of terrestrial plants (Hoagland and Arnon 1938). Complete nutrient solutions based on algal media (e.g. Wetzel and Manny 1972) clearly favor the growth of phytoplankton and attached algae while those based on hydroponic media (Gaudet 1963; Gerloff and Krombholz 1966; Stanley 1970; Basiouny, Garrard, and Haller 1977), in addition to promoting algal growth, bear little chemical resemblance to natural waters.

2. Recently, however, the earlier consideration of roots as merely organs of attachment (Brown 1913; Den Hartog and Segal 1964) has been questioned due to the morphological and functional similarities between roots of submersed macrophytes and those of terrestrial plants (Bristow 1975). Only within the past decade has the importance of nutrient uptake by roots of submersed macrophytes been fully realized (see review by Denny (1980)). This advance in our understanding of macrophyte nutrition facilitates the development of culture methodology employing solutions formulated specifically for growing submersed macrophytes on natural sediment.

Objective and scope

3. The objective of this report is to incorporate contemporary

information on the growth requirements of submersed macrophytes in the development of methodology for the laboratory culture and study of rooted submersed freshwater macrophytes. The information provided is intended to be of assistance in establishing and maintaining cultures of plants for experimental research, including physiological and ecological investigations, preliminary herbicide evaluations, and screening of microbial pathogens or herbivorous insects as potential weed control agents. Plant cultures may also be useful in providing mass quantities of physiologically or genetically similar plant tissues for subsequent observation or experimentation.

Culture Requirements

Nutrient sources

4. Although specific sites of nutrient uptake by submersed macrophytes may vary in relation to environmental (sediment/water) nutrient availability (Denny 1972; Patterson and Brown 1979; Barko 1982; Carignan 1982; Waisel, Agami, and Shapira 1982), it is now generally accepted that rooted submersed macrophytes can fulfill their phosphorus requirements by uptake from sediments (Bristow and Whitcombe 1971; DeMarte and Hartman 1974; Best and Mantai 1978; Bole and Allan 1978; Carignan and Kalff 1979, 1980; Welsh and Denny 1979a; Barko and Smart 1980, 1981a; Huebert and Gorham 1983). Recent studies have likewise demonstrated significant mobilization of nitrogen from sediments (Toetz 1974; Nicholls and Keeney 1976a, b; Best and Mantai 1978; Barko and Smart 1981a; Barko 1982; Huebert and Gorham 1983). Moreover, additions of nitrogen to solution, sediment, or both have not resulted in increased plant growth relative to unfertilized controls (Barko 1983; Barko and Smart 1983). Therefore, sufficient nitrogen and phosphorus to support adequate plant growth can usually be obtained from sediment in the absence of solution nitrogen or phosphorus.

5. While nitrogen and phosphorus are primarily acquired by root uptake from sediment, potassium appears, at least in some species, to be absorbed primarily from solution (Barko and Smart 1981a; Barko 1982). Although some sediments may provide sufficient potassium for moderate growth of submersed macrophytes, addition of potassium to solution usually stimulates growth and increases tissue potassium concentrations (Barko 1982; Huebert and Gorham 1983). To ensure optimal growth conditions, potassium should be included in culture solutions.

6. Calcium may be mobilized from sediment by some submersed macrophytes (DeMarte and Hartman 1974); however, *Potamogeton pectinatus* L. failed to grow in the absence of solution calcium (Huebert and Gorham 1983), and growth of *Myriophyllum spicatum* L. was reduced in solutions low in calcium (Barko 1982). The important role of calcium as a component of the carbonate system, in addition to its apparent involvement in photosynthetic bicarbonate utilization (Lowenhaupt 1956; Lucas and Dainty 1977), require that calcium be included in culture solution.

7. The relative importance of sediment and water as sources of magnesium, sodium, chloride, and sulfur for rooted submersed macrophytes is unclear. Reduced growth of *P. pectinatus* in solutions lacking magnesium (Huebert and Gorham 1983) suggests that magnesium may be acquired primarily from solution. Active foliar uptake of sulfate (SO_4) and chloride has been demonstrated in several rooted submersed macrophyte species (see review by Denny (1980)), indicating that these ions can be readily acquired from solution. However, omission of sulfate from solution did not affect the growth of *P. pectinatus* (Huebert and Gorham 1983), suggesting that sulfur can be obtained from sediment in the absence of solution sulfate. Similarly, both shoot and root uptake of sodium and chloride have been demonstrated in several species (Waisel, Agami, and Shapira 1982). Moreover, Shepherd and Bowling (1973) demonstrated active sodium and chloride uptake by roots of *Potamogeton natans*. Therefore, while magnesium may be required in culture solutions, plant demand for sodium, chloride, and sulfur apparently may be fulfilled by either root uptake from sediment or shoot uptake from solution. We generally include sodium, sulfate, and chloride in culture solutions because of their widespread occurrence in fairly high concentration in natural waters and as a convenient means of adding required amounts of bicarbonate, magnesium, and calcium, respectively.

8. Rooted aquatic plants are presumably able to satisfy their requirements for micronutrients by uptake from sediment (Huebert and Gorham 1983). Evidence for root uptake of iron (DeMarte and Hartman 1974; Gentner 1977; Basiouny, Haller, and Garrard 1977; Barko and Smart 1983), manganese (Barko and Smart 1983), copper (Welsh and Denny 1979b, 1980; Cushing and Thomas 1980), and zinc (Cushing and Thomas 1980) has been presented for some submersed freshwater macrophytes. The relative importance of root/shoot uptake of boron and molybdenum is unknown. We have observed no adverse effects attributable to the omission of boron, molybdenum, or other micronutrients from culture solutions,

and additions of boron, iron, manganese, zinc, and molybdenum to solution have not resulted in increased growth of *H. verticillata* (unpublished data) or *P. pectinatus* (Huebert and Gorham 1983).

Carbon supply and pH

9. Inorganic carbon sources in nature include dissolved inorganic carbon (DIC), CO_2 supplied by air/water exchange, and heterotrophic production of CO_2 by sediment and water-column respiration. Dissolved inorganic carbon should be included in culture solutions due to the importance of carbonate equilibria on chemical and biological processes in fresh waters (Stumm and Morgan 1981) and the ability of many submersed macrophyte species to utilize bicarbonate, in addition to free CO_2 , in photosynthesis (Raven 1970). Aeration should be provided to enhance the air/water exchange of CO_2 ; however, aeration alone cannot substitute for bicarbonate as a carbon source. We have been unable to obtain significant growth of either *H. verticillata* or *M. spicatum* in the absence of added bicarbonate in spite of vigorous aeration at ambient CO_2 levels.

10. Solution pH will be controlled by the levels of alkalinity and DIC. The use of organic buffers to artificially control pH is generally unnecessary and should be avoided due to possible toxicity of some buffers (Stanley 1970). While many species are adaptable to wide ranges in alkalinity, pH, and DIC, some species, generally those restricted to low pH/low alkalinity waters (Moyle 1945; Spence 1967; Hutchinson 1970; Seddon 1972; Hellquist 1980; Kadono 1982), may be unable to efficiently utilize bicarbonate in photosynthesis (Steemann Nielsen 1947; Kadono 1980; Allen and Spence 1981). These acidophilic species may require aeration with CO_2 -enriched air to achieve normal growth and metabolism. Augmenting the airstream CO_2 supply decreases pH and shifts carbonate equilibria to increase the proportion of free CO_2 ($\text{CO}_2 + \text{H}_2\text{CO}_3$) in solution.

Facilities

11. Vessels suitable for the culture of submersed macrophytes are available in a variety of inert materials. We have used white fiberglass-reinforced polyester tanks for a number of years and found them to be durable yet sufficiently lightweight for portability. Areal dimensions (1 to 2 m on a side in our laboratory) may vary depending on application and space availability, but the depth should approximate 1 m to accommodate the vertical growth of the cultured species. Larger or deeper vessels may be used but these are more costly, less convenient during planting and harvesting operations, and less portable. Deeper vessels may also produce significant shadowing problems under natural

lighting due to shading by the sidewalls. Culture vessels should be covered with a transparent material such as lucite to prevent the entry of dust and other airborne contaminants.

12. Aeration and mixing can be provided by airlifts constructed of plastic (polyvinyl chloride) pipe or other chemically inert material and fitted with air diffusers of the type used for aquarium aeration. Air may be provided by aquarium air pumps, but larger scale facilities may require a compressor and storage tank. To minimize evaporation from the culture solution, compressed air can be passed through a humidification column prior to introduction to the culture vessel.

13. Photosynthetically active radiation (PAR) levels between 300 and 1000 $\mu\text{E m}^{-2}\text{sec}^{-1}$ are suitable for the culture of most strictly submersed species (Bowes, et al. 1977; Titus and Adams 1979; Barko and Smart 1981b; Barko, Hardin, and Matthews 1982); however, floating-leaved species may benefit from higher levels (Barko, Hardin, and Matthews 1982). Room temperatures (20° - 25°C) are adequate for most species; however, some of the more subtropically occurring, exotic species (e.g. *H. verticillata*) may benefit from higher temperatures (Van, Haller, and Garrard 1978; Bowes, Holaday, and Haller 1979; Barko and Smart 1981b). Neutral density shade fabric may be used, if necessary, to reduce light intensity and solar heating in outdoor/glasshouse cultures. Heating can be simply and inexpensively provided with aquarium-type immersion heaters. However, in many cases it may be more convenient to employ liquid circulators, which provide both heating and cooling, while continuously circulating the culture solution (Barko and Smart 1981b).

Culture Development

Culture solutions

14. Culture solutions presented here were devised on the assumption that nitrogen, phosphorus, and micronutrients are obtained primarily by root uptake from sediments. The use of natural sediments as a nutrient substrate coupled with the omission of nitrogen and phosphorus from culture solutions largely prevent the occurrence of algal blooms in plant cultures, obviate the use of elaborate techniques for exenic culturing, and allow the use of culture solutions chemically more similar to natural waters than to artificial growth media. However, in some cases it may be necessary or desirable to axenically culture

plants on totally artificial media (Wetzel and McGregor 1968; Klaine and Ward 1981). Complete nutrient media which may be appropriate for axenic cultures are provided in Gerloff and Krombholz (1966), Bristow and Whitcombe (1971), and Wetzel and Manny (1972).

15. In many investigations, and for routine culture, it is desirable to provide a simple, yet near-optimal solution composition. For routine usage the general culture solution shown in Table 1 is easily formulated. Alternatively, some investigations may require the use of higher alkalinity solutions or solutions of more balanced composition (with respect to major cation and bicarbonate levels) to more closely approximate natural conditions. An important consideration relevant to the use of solutions high in calcium and bicarbonate is the increased likelihood of CaCO_3 precipitation resulting in changes in solution composition. An additional consideration is the resultant increased ash content of submersed macrophytes shoots due to CaCO_3 precipitation on leaf surfaces. We have attributed a slight reduction in growth of *M. spicatum* at elevated $\text{Ca}(\text{HCO}_3)_2$ levels to the development of a heavy precipitate on the leaves (unpublished data). Both of these problems may be ameliorated and plant growth increased by augmenting the airstream CO_2 concentration.

16. One of the problems of formulating moderate to high alkalinity solutions is the low solubility of calcium and magnesium carbonates and the unavailability of bicarbonate salts of these cations. Provision of carbon solely as sodium and potassium bicarbonates avoids the problem of solubility, but results in unnaturally high monovalent cation concentrations and atypical ratios among the major cations. For these reasons we prefer adding bicarbonate by simulating the natural weathering process of acid dissolution of CaCO_3 . We have accomplished complete solubility of added CaCO_3 by administering CO_2 to the solution prior to adding the required CaCO_3 . Dissolution is fairly rapid, but prolonged aeration with ambient air is required to subsequently equilibrate pCO_2 with atmospheric levels. This technique can be used to prepare solutions of various alkalinity and DIC levels such as the solution presented in Table 2 employing cation and bicarbonate proportions based on data obtained from alkaline lakes (Hutchinson 1957).

17. When preparing large quantities of solution it is desirable to measure alkalinity and pH as well as the concentrations of major components such as calcium and DIC to ensure that complete solubilization has occurred and that no gross errors or omissions of reagents have occurred. Equilibration

of the carbonate system can be verified from pH, DIC, and alkalinity (Stumm and Morgan 1981). Levels of DIC can be determined with an infrared gas analyzer or other carbon analyzer and calcium concentrations by atomic absorption spectrophotometry. However, in cases where these instruments are not readily available, and for rapid routine monitoring, ionic strength (an indicator of ionic composition) can be checked by measuring conductivity. We have used the relationship between conductance and ionic strength (Snoeyink and Jenkins 1980) to check the gross chemical composition of solutions spanning a sixfold range in alkalinity and major cation concentrations (Figure 1). Due to the strong influence of divalent cations on ionic strength, incomplete solubilization or precipitation of CaCO_3 can be readily detected. For example, failure to dissolve 10 mg l^{-1} calcium as CaCO_3 will result in a reduction in conductivity (25°C) of approximately $45 \mu\text{S cm}^{-1}$, while a similar loss due to precipitation of CaCO_3 will reduce conductivity approximately $35 \mu\text{S cm}^{-1}$.

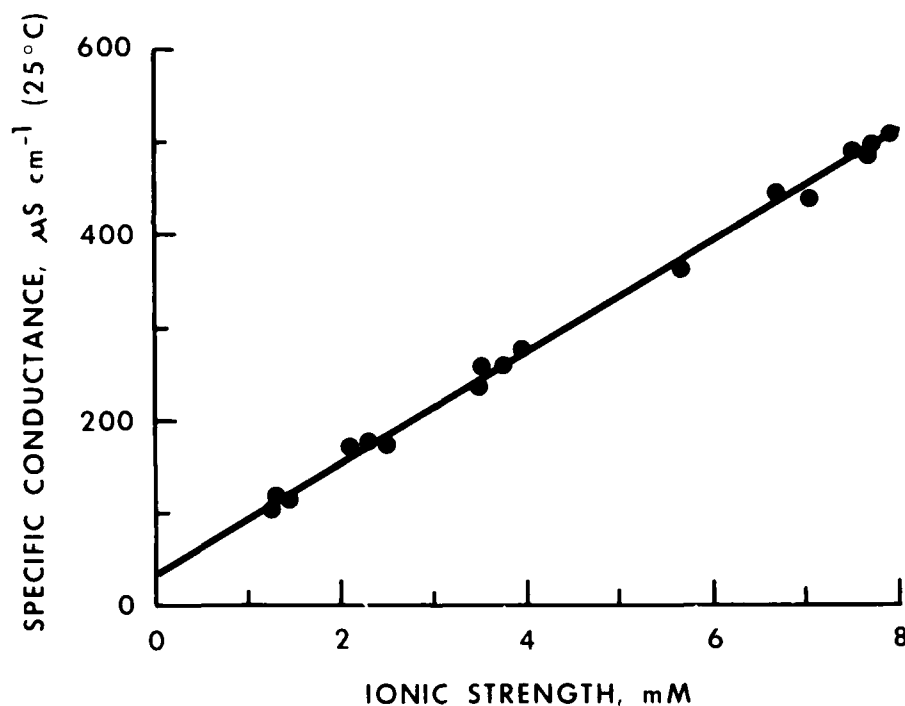


Figure 1. Relationship between specific conductance and ionic strength for 17 experimental solutions of various cation and bicarbonate concentrations

Sediment substrate

18. We have obtained maximum yields of a variety of submersed aquatic plant species on fine-textured lake sediments with an organic content of less

than 15 percent dry weight. Sediments containing higher levels of refractory organic matter or a substantial (>75 percent) sand-sized fraction are generally poor substrates for submersed aquatic plants (Barko and Smart, subm.) However, on fine-textured, mineral sediments we frequently obtain yields equivalent to $\sim 1200 \text{ g dry wt m}^{-2}$ sediment surface area.

19. It should be noted that commercially available potting soils are generally unsuitable substrates for submersed aquatic plant growth. Cultures initiated on several types of organic potting soils exhibited greatly reduced growth, chlorosis, or death (personal observation), perhaps due to the anaerobic formation of inhibitory organic compounds (Harper and Lynch 1982; Barko and Smart 1983). In addition many potting soil mixtures contain low-density components (perlite, vermiculite, or peat), which are buoyant, resulting in undesirable turbidity and, ultimately, algal blooms in the culture solution.

20. Peat-sand or muck-sand mixtures have been frequently used for culturing and experimental studies of rooted submersed macrophytes in flow-through systems (Steward and Center 1979; Langeland and Sutton 1980; Sutton, Littell, and Langeland 1980; Westerdahl and Hall 1983); however, the use of these relatively infertile substrates may result in nutrient limitation (Langeland, Sutton, and Canfield 1983; Hall, Westerdahl, and Stewart 1984). Plant growth has also been shown to be considerably reduced on peat-sand relative to that obtained on fine-textured lake sediment (Hall, Westerdahl, and Stewart 1984). These problems might be alleviated by addition of nutrients to the water or substrate; however, nutrient enrichment may result in excessive growth of phytoplankton and attached algae (Mulligan and Baranowski 1969; Ryan, Reimer, and Toth 1972; Mulligan, Baranowski, and Johnson 1976).

21. Sediment for plant culture can be obtained by dredging and should be allocated to planting containers several days prior to use to allow for settling. Sediment containers providing a depth of 10 to 20 cm and a volume of 1 to 4 l are suitable for most species. Approximately 2 cm should be allowed for placement of a layer of coarse silica sand over the sediment to minimize physical exchanges between the sediment and the culture solution (Hynes and Grieb 1970).

Plant propagation

22. We have used different methods of plant propagation depending upon the growth form of the species selected for culture. Many species can be rooted from apical cuttings. Fresh apical cuttings 10 to 15 cm in length are taken from healthy, nonflowering stems and inserted to a depth of about 5 cm into the

sediment. Root formation generally begins within 1 week. Stocks of plant species amenable to this method of propagation can be rapidly expanded by repeated subculturing.

23. Some species produce underground vegetative organs (tubers/rhizomes) (Sculthorpe 1967; Van, Haller, and Garrard 1978) or specialized apices (turions) (Sculthorpe 1967; Sastroutomo 1980, 1981) which can be obtained directly from the field or from biological suppliers. Vegetative propagules of the above types can usually be stored in a refrigerator between layers of moist toweling for several months until needed. Species which readily reproduce by stolons or runners are best planted as intact plants (Sculthorpe 1967). Many species can be propagated from seed in addition to the above methods. Additional information relevant to propagation has been reviewed by Sculthorpe (1967).

Culture Maintenance

Subculturing

24. After prolonged periods of growth, cultures of submersed macrophytes lose viability, either due to the onset of senescence or to the depletion of sediment or water resources. In order to maintain viable cultures over long periods, the investigator must periodically initiate subcultures from secondary cuttings. The frequency of subculturing will depend on rates of growth and phenological development; therefore, cultures maintained at higher temperatures will require more frequent subculturing to prevent deterioration (Barko and Smart 1981b). Some species (notably *M. spicatum*) are prone to flowering and subsequent senescence soon after developing a canopy at the water surface (Grace and Wetzel 1978; Barko and Smart 1981b). The investigator should observe plant cultures frequently and initiate subcultures prior to the onset of flowering since flowering apices generally exhibit a greatly reduced ability to form roots.

25. Subcultures should be initiated on fresh sediment, as we have repeatedly observed diminished growth of several species on previously planted sediments. Whether this diminished growth is due to nutrient depletion, accumulation of toxins, or some other mechanism is unclear and is the subject of current investigation.

Algal control

26. During short-term experiments (6 to 8 weeks), the development of

algal populations in solutions lacking nitrogen and phosphorus is usually minor. However, in mass cultures subjected to repeated clipping, or in extended experiments involving some degree of tissue senescence or damage, algal populations may reach undesirable levels. Phytoplankton can be largely controlled through the use of diatomaceous earth filters of the type commonly used for aquariums. This method of control is favored as it minimizes disturbance of the plants, does not require toxic chemicals, minimizes chemical changes in the solution, and effectively removes nitrogen and phosphorus incorporated into the algal cells, thus reducing the probability and severity of subsequent algal blooms. The use of algicides such as CuSO_4 should be avoided as these may be toxic to aquatic plants even in low concentrations (Ryan and Riemer 1975).

Maintenance of solution composition

27. One of the major differences between a culture of submersed macrophytes and a field population is a greatly reduced rate of water-column and sediment respiration in the former. For this reason, carbon supplied as DIC to macrophyte cultures may be inadequate to support photosynthetic carbon requirements. While aeration provides a major source of inorganic carbon, we have observed significant reductions in DIC and subsequent increases in pH due to plant uptake of DIC. Reduction in DIC may also result from precipitation of CaCO_3 . Increasing the air/water exchange of CO_2 by increasing the aeration rate or by augmenting the CO_2 concentration in the air stream should ameliorate the above problems. Changes in solution composition due to reduction in DIC can also be lessened by using low calcium, low alkalinity solutions such as the general culture solution described here; however, in this case it should be realized that most of the carbon required for plant growth must be provided through aeration.

28. Another major difference between a culture of submersed plants and a field population is a reduced rate of water exchange (particularly in static cultures). Changes in chemical composition of culture solutions are ultimately dependent on the ratio of solution volume to plant biomass. In productive systems in the temperate zone, submersed macrophytes may attain a biomass of $500 \text{ g dry wt m}^{-2}$ (Westlake 1975) in a water column of about 2 m in depth (2000 l m^{-2}). These figures provide a minimal solution volume:biomass ratio of 4 l g^{-1} (ignoring water exchange). However, for experimental cultures, we recommend a solution volume:biomass ratio of 10 or higher to avoid substantial changes in solution composition. The use of the largest practical culture

vessels with sediment containers occupying ≤ 10 percent of the bottom surface area minimizes crowding of plants and sediment-water exchange of nitrogen and phosphorus, and generally provides a sufficiently high solution:biomass ratio. Low volume systems may require continuous flow (Westerdahl and Hall 1983) or partial solution changes (Barko 1982) to avoid substantial changes in solution composition. For routine culturing, solution volume:biomass ratios can be increased and sediment containers can occupy 50 percent of the bottom surface area of the culture vessel. In this case, changes in solution composition are minimized by the use of low calcium, low alkalinity solutions, and the frequent initiation of subcultures in fresh solution.

Experimental Use of Cultures

29. One objective of submersed macrophyte culturing may be direct experimentation (i.e. experimental manipulation of culture conditions). An important consideration in the experimental use of laboratory cultures is to provide near-natural conditions with respect to nonexperimental parameters while investigating macrophyte responses to specific, experimentally manipulated variables. Experimental investigations should be designed to maximize biomass increase, thereby ensuring that plant biomass, tissue nutrient concentrations, and physiological state of plant tissues reflect experimental conditions rather than those the plants were exposed to prior to investigation. Under optimal solution, substrate, light, and temperature conditions, sufficient plant growth can be obtained in 5 to 6 weeks. Longer experimental durations may be required in some cases, but some species exhibit a tendency to slough senescent tissues, contributing to the development of algal problems as well as obfuscating treatment-related differences in growth (Barko and Smart 1981b). An additional consideration in longer experiments is that plant growth may ultimately be limited by variables (light, space, carbon, nutrients, etc.) not chosen for study. Under such conditions treatment-related differences in plant growth may decrease over time as plant populations approach maximal biomass attainable under a given set of environmental conditions.

30. Another possible objective of plant culturing may be to provide mass quantities of physiologically or genetically similar plant tissues for subsequent observation or experimentation. These tissues, cultured under near-natural but controlled conditions, may be more suitable for physiological or

morphological study than those collected from temporally and spatially varying natural environments. An additional advantage to this approach is that plant tissues of a variety of species can be made available for laboratory study throughout the year or in areas remote from natural macrophyte populations.

References

- Allen, E. D. and Spence, D. H. N. 1981. The differential ability of aquatic plants to utilize the inorganic carbon supply in fresh waters. *New Phytol.* 87:269-283.
- Barko, J. W. 1982. Influence of potassium source (sediment vs. open water) and sediment composition on the growth and nutrition of a submersed freshwater macrophyte (*Hydrilla verticillata* (L.f.) Royle). *Aquat. Bot.* 12:157-172.
- Barko, J. W. 1983. The growth of *Myriophyllum spicatum* L. in relation to selected characteristics of sediment and solution. *Aquat. Bot.* 15:91-103.
- Barko, J. W. and Smart, R. M. 1980. Mobilization of sediment phosphorus by submersed freshwater macrophytes. *Freshwater Biol.* 10:229-238.
- Barko, J. W. and Smart, R. M. 1981a. Sediment-based nutrition of submersed macrophytes. *Aquat. Bot.* 10:339-352.
- Barko, J. W. and Smart, R. M. 1981b. Comparative influences of light and temperature on the growth and metabolism of selected submersed freshwater macrophytes. *Ecol. Monogr.* 51:219-235.
- Barko, J. W. and Smart, R. M. 1983. Effects of organic matter additions to sediment on the growth of aquatic plants. *J. Ecol.* 71:161-175.
- Barko, J. W. and Smart, R. M. Subm. Sediment organic matter as an index for growth of rooted submersed aquatic vegetation. *Limnol. Oceanogr.*
- Barko, J. W., Hardin, D. G. and Matthews, M. S. 1982. Growth and morphology of submersed freshwater macrophytes in relation to light and temperature. *Can. J. Bot.* 60:877-887.
- Basiouny, F. M., Garrard, L. A. and Haller, W. T. 1977. Absorption of iron and growth of *Hydrilla verticillata* (L.f.) Royle. *Aquat. Bot.* 3:349-356.
- Basiouny, F. M., Haller, W. T. and Garrard, L. A. 1977. Evidence for root iron nutrition in *Hydrilla verticillata* Royle. *Pl. Soil* 48:621-627.
- Best, M. D. and Mantai, K. E. 1978. Growth of *Myriophyllum*: sediment or lake water as the source of nitrogen and phosphorus? *Ecology* 59:1075-1080.
- Bole, J. B. and Allan, J. R. 1978. Uptake of phosphorus from sediment by aquatic plants *Myriophyllum spicatum* and *Hydrilla verticillata*. *Water Res.* 12:353-358.
- Bourn, W. S. 1932. Ecological and physiological studies on certain aquatic angiosperms. *Contr. Boyce Thompson Inst.* 4:425-496.
- Bowes, G., Holaday, A. S. and Haller, W. T. 1979. Seasonal variation in the biomass, tuber density, and photosynthetic metabolism of hydrilla in three Florida lakes. *J. Aquat. Plant Manage.* 61-65.

- Bowes, G., Van, T. K., Garrard, A. and Haller, W. T. 1977. Adaptation to low light levels by *Hydrilla*. J. Aquat. Plant Manage. 15:32-35.
- Bristow, J. M. 1975. The structure and function of roots in aquatic vascular plants. In: J. G. Torrey and D. T. Clarkson (Editors), The Development and Function of Roots. Academic Press, New York, N.Y., pp 221-233.
- Bristow, J. M. and Whitcombe, W. 1971. The role of roots in the nutrition of aquatic vascular plants. Amer. J. Bot. 58:8-13.
- Brown, W. H. 1913. The relation of the substratum to the growth of *Elodea*. Philippine J. Sci. 8:1-20.
- Carignan, R. 1982. An empirical model to estimate the relative importance of roots in phosphorus uptake by aquatic macrophytes. Can. J. Fish. Aquat. Sci. 39:243-247.
- Carignan, R. and Kalff, J. 1979. Quantification of the sediment phosphorus available to aquatic macrophytes. J. Fish. Res. Bd. Can. 36:1002-1005.
- Carignan, R. and Kalff, J. 1980. Phosphorus sources for aquatic weeds: water or sediment? Science 207:987-989.
- Cushing, C. E., Jr. and Thomas, J. M. 1980. Cu and Zn kinetics in *Myriophyllum heterophyllum* Michx. and *Potamogeton richardsonii* (Ar. Benn.) Rydb. Ecology 61:1321-1326.
- DeMarte, J. A. and Hartman, R. T. 1974. Studies on absorption of ^{32}P , ^{59}Fe , and ^{45}Ca by water-milfoil (*Myriophyllum exalbescens* Fernald). Ecology 55:188-194.
- Den Hartog, C. and Segal, S. 1964. A new classification of the water plant communities. Acta Botanica Neerl. 13:367-393.
- Denny, P. 1972. Sites of nutrient absorption in aquatic macrophytes. J. Ecol. 60:819-829.
- Denny, P. 1980. Solute movement in submerged angiosperms. Biol. Rev. 55:65-92.
- Forsberg, C. 1965. Nutritional studies of *Chara* in axenic cultures. Physiol. Planta. 18:275-290.
- Gaudet, J. J. 1963. *Marsilea vestita*: Conversion of the water form to the land form by darkness and by far-red light. Science 140:975-976.
- Gentner, S. R. 1977. Uptake and transport of iron and phosphate by *Vallisneria spiralis* L. Aquat. Bot. 3:267-272.
- Gerloff, G. C. and Krombholz, P. H. 1966. Tissue analysis as a measure of nutrient availability for the growth of angiosperm aquatic plants. Limnol. Oceanogr. 11:529-537.
- Grace, J. B. and Wetzel, R. G. 1978. The production biology of Eurasian watermilfoil (*Myriophyllum spicatum* L.): a review. J. Aquat. Plant Manage. 16:1-11.
- Hall, J. F., Westerdahl, H. E. and Stewart, T. J. 1984. Growth response of *Myriophyllum spicatum* and *Hydrilla verticillata* when exposed to continuous, low concentrations of fluridone. Tech. Rept. A-84-1. US Army Engineer Waterways Experiment Station, Vicksburg, Miss.

- Harper, S. H. T. and Lynch, J. M. 1982. The role of water-soluble components in phytotoxicity from decomposing straw. *Pl. Soil* 65:11-17.
- Hellquist, C. B. 1980. Correlation of alkalinity and the distribution of *Potamogeton* in New England. *Rhodora* 82:331-344.
- Hoagland, D. R. and Arnon, D. I. 1938. The water-culture method for growing plants without soil. *Calif. Agric. Exp. Stn. Circ.* 347, 32 pp.
- Huebert, D. B. and Gorham, P. R. 1983. Biphasic mineral nutrition of the submersed aquatic macrophyte *Potamogeton pectinatus* L. *Aquat. Bot.* 16:269-284.
- Hutchinson, G. E. 1957. A Treatise on Limnology. Vol 1. John Wiley and Sons, New York. 1015 pp.
- Hutchinson, G. E. 1970. The chemical ecology of three species of *Myriophyllum* (Angiospermae, Haloragaceae). *Limnol. Oceanogr.* 15:1-5.
- Hynes, H. B. N. and Grieb, B. J. 1970. Movement of phosphate and other ions from and through lake muds. *J. Fish. Res. Board Can.* 27:653-668.
- Imahori, K. and Iwasa, K. 1965. Pure culture and chemical regulation of the growth of Charophytes. *Phycologia* 4:127-134.
- Kadono, Y. 1980. Photosynthetic carbon sources in some *Potamogeton* species. *Bot. Mag. Tokyo* 93:185-194.
- Kadono, Y. 1982. Occurrence of aquatic macrophytes in relation to pH, alkalinity, Ca^{++} , Cl^- and conductivity. *Jap. J. Ecol.* 32:39-44.
- Klaine, S. J. and Ward, C. H. 1981. Axenic culture of hydrilla. *J. Aquat. Plant Manage.* 19:59-60.
- Langeland, K. A. and Sutton, D. L. 1980. Regrowth of hydrilla from axillary buds. *J. Aquat. Plant Manage.* 18:27-29.
- Langeland, K. A., Sutton, D. L. and Canfield, D. E., Jr. 1983. Growth response of hydrilla to extractable nutrients in prepared substrates. *J. Freshwater Ecol.* 2:263-272.
- Lowenhaupt, B. 1956. The transport of calcium and other cations in submerged aquatic plants. *Biol. Rev.* 31:371-395.
- Lucas, W. J. and Dainty, J. 1977. HCO_3^- influx across the plasmalemma of *Chara corallina*: divalent cation requirement. *Plant Physiol.* 60:862-867.
- Moyle, J. B. 1945. Some chemical factors influencing the distribution of aquatic plants in Minnesota. *Amer. Midl. Natur.* 34:402-420.
- Mulligan, H. F. and Baranowski, A. 1969. Growth of phytoplankton and vascular aquatic plants at different nutrient levels. *Verh. Internat. Verein. Limnol.* 17:802-810.
- Mulligan, H. F., Baranowski, A. and Johnson, R. 1976. Nitrogen and phosphorus fertilization of aquatic vascular plants and algae in replicated ponds. (1) Initial response to fertilization. *Hydrobiol.* 48:109-116.
- Nichols, D. S. and Keeney, D. R. 1976a. Nitrogen nutrition of *Myriophyllum spicatum*: variation of plant tissue nitrogen concentration with season and site in Lake Wingra. *Freshwater Biol.* 6:137-144.

- Nichols, D. S. and Keeney, D. R. 1976b. Nitrogen nutrition of *Myriophyllum spicatum*: uptake and translocation of ^{15}N by shoots and roots. *Freshwater Biol.* 6:145-154.
- Patterson, K. J. and Brown, J. M. A. 1979. Growth and elemental composition of the aquatic macrophyte, *Lagarosiphon major*, in response to water and substrate nutrients. *Prog. Water Technol.* 11:231-246.
- Pringsheim, E. G. and Pringsheim, O. 1962. Axenic culture of *Utricularia*. *Amer. J. Bot.* 49:898-901.
- Raven, J. A. 1970. Exogenous inorganic carbon sources in plant photosynthesis. *Biol. Rev.* 45:167-221.
- Ryan, J. B. and Reimer, D. N. 1975. Copper toxicity symptoms in Sago pondweed (*Potamogeton pectinatus* L.). *Proc. NE Weed Sci. Soc.* 19:108-113.
- Ryan, J. B., Reimer, D. N. and Toth, S. J. 1972. Effects of fertilization on aquatic plants, water, and bottom sediments. *Weed Sci.* 20:482-486.
- Sastroutomo, S. S. 1980. Dormancy and germination in axillary turions of *Hydrilla verticillata*. *Bot. Mag. Tokyo* 93:265-273.
- Sastroutomo, S. S. 1981. Turion formation, dormancy and germination of curly pondweed, *Potamogeton crispus* L. *Aquat. Bot.* 10:161-173.
- Schulthorpe, C. D. 1967. *The Biology of Aquatic Vascular Plants*. Edward Arnold, London, 610 pp.
- Seddon, B. 1972. Aquatic macrophytes as limnological indicators. *Freshwat. Biol.* 2:107-130.
- Shepard, V. H. and Bowling, D. J. F. 1973. Active accumulation of sodium by roots of five aquatic species. *New Phytol.* 72:1075-1080.
- Snoeyink, V. L. and Jenkins, D. 1980. *Water Chemistry*. John Wiley and Sons, New York, 463 pp.
- Spence, D. H. N. 1967. Factors controlling the distribution of freshwater macrophytes with particular reference to the lochs of Scotland. *J. Ecol.* 55:147-170.
- Stanley, R. A. 1970. Studies on nutrition, photosyntheses and respiration in *Myriophyllum spicatum* L. Ph.D. Dissertation, Duke University, Durham, N. C.
- Steemann Nielsen, E. 1947. Photosynthesis of aquatic plants with special reference to the carbon sources. *Dansk Botanisk Arkiv.* 12:1-71.
- Steward, K. K. and Center, T. D. 1979. Evaluation of metham for control of hydrilla regrowth from tubers. *J. Aquat. Plant Manage.* 17:76-77.
- Stumm, W. and Morgan, J. J. 1981. *Aquatic Chemistry*. John Wiley and Sons, New York, 780 pp.
- Sutton, D. L., Littell, R. C. and Langeland, K. A. 1980. Intraspecific competition of *Hydrilla verticillata*. *Weed Sci.* 28:425-428.
- Titus, J. E. and Adams, M. S. 1979. Coexistence and the comparative light relations of the submersed macrophytes *Myriophyllum spicatum* L. and *Vallisneria spiralis* Michx. *Oecologia* 40:273-286.
- Toetz, D. W. 1974. Uptake and translocation of ammonia by freshwater hydrophytes. *Ecology* 55:199-201.

- Van, T. K., Haller, W. T. and Garrard, L. A. 1978. The effect of day-length and temperature on hydrilla growth and tuber production. *J. Aquat. Plant Manage.* 16:57-59.
- Waisel, Y., Agami, M. and Shapira, Z. 1982. Uptake and transport of ^{86}Rb , ^{32}P , ^{36}Cl , and ^{22}Na by four submerged hydrophytes. *Aquat. Bot.* 13:179-186.
- Welsh, R. P. H. and Denny, P. 1979a. The translocation of ^{32}P in two submerged aquatic angiosperm species. *New Phytol.* 82:645-656.
- Welsh, R. P. H. and Denny, P. 1979b. Translocation of lead and copper in two submerged angiosperm species. *J. Exp. Bot.* 30:339-345.
- Welsh, R. P. H. and Denny, P. 1980. The uptake of lead and copper by submerged aquatic macrophytes in two English Lakes. *J. Ecol.* 68.
- Westerdahl, H. E. and Hall, J. F. 1983. Threshold 2,4-D concentrations for control of Eurasian watermilfoil and sago pondweed. *J. Aquat. Plant Manage.* 21:22-25.
- Westlake, D. F. 1975. Primary production of freshwater macrophytes. In: J. P. Cooper (Editor), *Photosynthesis and Productivity in Different Environments*. Cambridge University Press, Cambridge, pp 189-206.
- Wetzel, R. G. and Manny, B. A. 1972. Secretion of dissolved organic carbon and nitrogen by aquatic macrophytes. *Verh. Internat. Verein. Limnol.* 18:162-170.
- Wetzel, R. G. and McGregor, D. L. 1968. Axenic culture and nutritional studies of aquatic macrophytes. *Amer. Midl. Natur.* 80:52-64.

Table 1
Chemical Composition and Formulation of a General Purpose
Culture Solution

Chemical Composition		Formulation	
Parameter	Concentration	Reagent	Quantity, mg ℓ^{-1}
Ca, mg ℓ^{-1}	25.0	$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	91.7
Mg, mg ℓ^{-1}	6.8	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	69.0
Na, mg ℓ^{-1}	16.0	NaHCO_3	58.4
K, mg ℓ^{-1}	6.0	KHCO_3	15.4
DIC, mg ℓ^{-1}	10.2		
SO_4 , mg ℓ^{-1}	26.9		
Cl, mg ℓ^{-1}	44.2		
Alkalinity, meq ℓ^{-1}	0.85		
Ionic strength, mM	3.9		
Conductivity, $\mu\text{S cm}^{-1}$ (25°C)	280.0		
pH (air equilibrium)	7.9		

Table 2
Chemical Composition and Formulation of an Ionically Balanced,
Alkaline Solution*

Chemical Composition		Formulation	
Parameter	Concentration	Reagent	Quantity, mg ℓ^{-1}
Ca, mg ℓ^{-1}	60.0	$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	91.6
Mg, mg ℓ^{-1}	10.1	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	102.3
Na, mg ℓ^{-1}	16.8	Na_2SO_4	52.0
K, mg ℓ^{-1}	6.1	K_2SO_4	13.6
DIC, mg ℓ^{-1}	21.0	CaCO_3^{**}	87.5
SO_4 , mg ℓ^{-1}	82.5		
Cl, mg ℓ^{-1}	44.2		
Alkalinity, meq ℓ^{-1}	1.75		
Ionic strength, mM	7.5		
Conductivity, $\mu\text{S cm}^{-1}$ (25°C)	490.0		
pH (air equilibrium)	8.3		

* Cation and inorganic carbon proportions are based on data presented for alkaline lakes (Hutchinson 1957).

** Requires addition of CO_2 gas to achieve solubility. Note that approximately 50 percent of the final DIC concentration is derived from gaseous CO_2 .

END

FILMED

9-85

DTIC

